ISSN: 0975 – 8704
IJBLST (2016), 8(3):16-22

Antimicrobial Effect of Some Spices on Storage Moulds of Cocoa Beans in South-western Nigeria

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Abstract
The proliferation of moulds in stored cocoa beans had been a major challenge in Nigeria's cocoa industry, a problem which needs to be tackled with the aid of non-toxic, natural and environmental friendly materials. The anti-microbial efficacies of aqueous extracts as well as powdered forms of some spices- *Aframomum danielli, A. melegueta* and *Tetrapleura tetraptera* were tested against three most occurring toxigenic cocoa beans storage moulds in Southwest Nigeria: *Aspergillus flavus, A. ochraceous* and *A. versicolor*. The average percent inhibitions exhibited against the three test fungi by hot water extracts of *A. danielli, A. melegueta* and *T. tetraptera* ranged between 22.95 – 29.64%, 34.42 – 41.38% and 3.43 – 14.89% respectively. Cold water extracts of *A. danielli* exhibited the highest inhibitions (10.18 – 33.94%) against the organisms while that of *T. tetraptera* (0.79 – 10.71%) produced the least. Powdered forms of the three botanicals as well as their combinations however exhibited higher average percent inhibitions (4.46 – 70.44%) against the test organisms. Findings from this study have revealed that hot water extracts of *A. melegueta* were more effective against *A. flavus, A. versicolor* and *A. ochraceous* than their cold water extracts. Combinations of at least two of the powdered forms of the botanicals, though not synergistic, were also able to effect some appreciable degree of control against the fungi. In addition to proper handling of cocoa beans before and during storage, powdered forms of *A. danielli* and *A. melegueta* can therefore be used to effectively control mouldiness of cocoa beans in storage.

Key words: Efficacy, cocoa beans, moulds, storage, botanicals

Introduction

Theobroma cacao, a native to the Americas is one major perennial tropical crop of economic importance [1]. Its beans are of commercial, nutritional and medical importance to man. The seasonal production of cocoa makes its storage essential over a long period [2].

During storage, cocoa beans are associated with certain parasitic organisms. These include storage insect pests (*Araecerus fasciculatus*, *Ephestia cautella* (tropical warehouse moth), and *Tribolium castaneum*) and moulds (fungi) - *Aspergillus niger, Aspergillus flavus, Fusarium spp., Neurospora spp. Penicillium spp.*, etc.. The insects are capable of feeding directly on the beans (thereby causing loss of weight of the commodity), boring into the beans thereby stripping the beans of their natural defence mechanism, attracting moths' larva and predisposing the beans to rot fungi [3], [4]. Spores of moulds require moisture to germinate on or within various stored agricultural products and if this is excluded (i.e. if grain is stored at its safe moisture content or below) moulds generally will not grow. The maximum safe storage moisture content may be defined as the amount of absorbed water held within a commodity which is in equilibrium with an atmospheric relative humidity of 70%. Moisture content is closely bound to temperature. Under certain circumstances, temperature differences can cause the re-distribution of moisture leading to local mould growth. Other factors that affect fungal development and the production of spores are availability of oxygen, light, acidity, and salt/sugar content [5].

However, the continual growth and proliferation of storage moulds on stored agricultural products if unabated, is capable of causing a huge loss to the farmer beyond a bearable limit. To this end, a number of chemicals had been employed to checkmate the microorganisms. Microbial control in foods could be assured by suppressing one or more essential factors for microbial survival [6].

Although effective, the use of synthetic chemicals is being discouraged due to their undesirable harmful effects on human health and the environment. The use of plant extracts (one of the non-chemical control alternatives) is however gradually gaining ground due to their availability,

ISSN: 0975 – 8704
IJBLST (2016), 8(3):16-22

non-toxicity and friendliness to the environment [7].

Antifungal activity of spices and derivatives has been studied regarding viable cell counts, mycelial growth and mycotoxins synthesis. In a study carried out to determine the effectiveness of nine essential oils to control the growth of mycotoxin-producing moulds and it was discovered that clove, cinnamon and oregano were able to prevent the growth of *Aspergillus parasiticus* and *Fusarium moniliforme*, while clove (ground and essential oil) markedly reduced the aflatoxin synthesis in infected grains [8]. These findings could be useful for rural communities to prevent the synthesis of fungal toxins in contaminated grains by simple measures. Where plants are used as storage protectants, they are almost always applied to control insect pests.

In agreement with the fungal inhibitory properties of spices, aqueous extracts of weeds have also been shown to inhibit toxin production by *Aspergillus flavus*. Both fresh and dried plant materials/parts (such as rhizomes, roots, stems, barks, seeds and fruits) have been examined and used in the control of pests [9], [10].

In view of the above, this study seeks to study the antimicrobial effects of powdered forms and aqueous extracts of some spices- *Aframomum danielli, A. melegueta* and *Tetrapleural tetraptera* against some cocoa beans storage moulds isolated in Southwest Nigeria.

Methodology

Pure cultures of each of three most occurring and highly toxigenic moulds- *Aspergillus flavus, A. ochraceous* and *A. versicolor* isolated from infected stored cocoa beans in Southwest Nigeria [11] as obtained from Plant Pathology Section, Cocoa Research Institute of Nigeria, were used for the bioassay of aqueous and powdered forms of some commonly found spices in the region. These included *Aframomum melegueta, A. danielli* and *Tetrapleura tetraptera*. The botanicals were prepared as described below.

Preparation of hot water extracts

Dried seeds of *Aframomum melegueta* and *Aframomum danielli* as well as the pods (fruits) of *Tetrapleura tetraptera* were ground separately into fine particles (about 40µm) with the aid of sterile mortar and pestle. Exactly 100g of each of the ground samples were transferred into 200ml sterile distilled water and boiled at 80°C for 10 minutes. The mixture was well homogenized and filtered through three layers of muslin cloth. Filtrate obtained from each homogenized sample was sterilized at 121°C and 1.1 kg cm^{-2} for 15 minutes. Each of the sterilized extracts was considered as a stock.

Preparation of cold water extracts

One hundred grams of each dried seeds/pods were ground separately with the aid of sterile mortar and pestle into fine particles and soaked in 200ml sterile distilled water for 48 hours. The soaked extracts were homogenized, filtered and sterilized as in the case of the hot water extraction. Each of the sterilized extracts was taken as the stock.

Bioassay of the plant extracts

Different volumes (2, 4 and 8ml) of each of the prepared aqueous extract stocks were introduced and thoroughly mixed (using poisoned food technique) into 8, 16 and 12ml sterilized PDA medium respectively to produce 10, 20 and 40% concentrations (Gautum *et al.*, 2012). Ground powders (1g, 2g and 4g) of each of the three dried spices were sterilized at 80°C in an oven for 2hours and incorporated (using poisoned food technique) into 19, 18 and 16ml sterilized and cooled (45°C) PDA respectively to give 5, 10 and 20% concentrations. At least two of each of the ground powdered spices were also combined together in equal proportions and then incorporated into already sterilized PDA. Small disk of pure culture of each of the test fungi isolates (*A. flavus, A. versicolor* and *A. ochraceous*) was cut with the aid of sterile cork borer No. 6 (10mm diameter) and aseptically transferred into the centre of a Petri dish containing the medium with different extract concentration. The plates were incubated at ambient temperature and growth diameter of each of the inoculated plates was taken every 24 hours until the mycelia growth of fungus covered the whole surface of Petri plates in control treatment. The percentage mycelial growth inhibitions were calculated using the formula:

$$\frac{d_c - d_t}{d_c} \times 100 \qquad \text{(Eqn. 1)}$$

Where: d_c = Mycelia growth diameter in control
d_t = Mycelia growth diameter in treatment

ISSN: 0975 – 8704
IJBLST (2016), 8(3):16-22

Results obtained from the work were subjected to Analysis of Variance (ANOVA) and the means separated using Fisher's Least Significant Difference (LSD) test at 5% level of probability and with the aid of Statistical Analysis System (SAS) 9.1 statistical package. The analysed results were also represented with charts for better clarity.

Results

Figure 1 shows details of mycelia growth inhibition of the three test fungi isolates by different concentrations of hot water extracts of the three spices used. The data revealed that hot water extracts of *A. danielli* was able to exhibit percent growth inhibitions of between 7.60 – 38.17% against *A. flavus,* while similar extracts of *A. melegueta* and *T. tetraptera* produced percent inhibition ranges of 34.32 – 50.14% and 10.73 – 18.58% respectively. Hot water extracts of *A. melegueta* at 40% concentration produced the highest inhibition (50.14%) against the organism, followed by 20% concentration of the same botanical (39.68%) and 40% *A. danielli* concentration (38.17%), which were not significantly different (P ≤ 0.05) from each other. The lowest growth inhibitions induced against the fungus were 10.73 and 7.60% (by 10% concentrations of *T. tetraptera* and *A. danielli* respectively).

The percent inhibition exhibited by hot water extracts of the three botanicals used in this study against *A. versicolor* ranged between 17.23 – 28.69% (*A. danielli*), 35.60 – 44.75% (*A. melegueta*) and 1.72 – 5.30% (*T. tetraptera*). The highest inhibitions (44.75 and 44.27/%) were exhibited by 40 and 20% concentrations of *A. melegueta,* followed by the 10% concentration of the same spice which produced 35.60% inhibition. Similarly, 40% hot water extract of *A. melegueta* exhibited the highest inhibition, 42.38% against *A. ochraceous*, followed by 40% *A. danielli* and 20% *A. melegueta* which produced 37.97 and 33.70% inhibitions respectively. *Tetrapleural tetraptera* hot water extracts at 10, 20 and 40% concentrations exhibited the lowest percent inhibitions of 3.96, 5.33 and 8.14% respectively.

Cold water extracts of *A. danielli, A. melegueta* and *T. tetraptera* exhibited percent mycelial inhibitions of between 17.92 – 40.45%, 16.28 – 34.05% and 4.24 – 8.59% respectively against *A. flavus.* The highest percent inhibition of the fungus mycelia (40.45%) was exhibited by 40% concentration of *A. danielli.* This inhibition was however not significantly different (P ≤ 0.05) from those produced by 20% *A. danielli* (36.25%) and 40% *A. melegueta* (34.05%). Significantly lowest inhibition (P ≤ 0.05) was however exhibited by *T. tetraptera* at 10% concentration (Figure 2).

While the percent growth inhibitions exhibited by *A. danielli, A. melegueta* and *T. tetaptera* against *A. versicolor* ranged between 3.02 – 13.20%, 3.01 – 16.92% and 0.00 – 2.36% respectively, the percent mycelial growth inhibition of *A. ochraceous* as exhibited by the same set of extracts ranged between 28.82 – 38.61%, 14.00 – 28.39% and 8.99 – 11.66% respectively. The highest inhibitions recorded on *A. versicolor* (16.92%) and *A. ochraceous* (38.61%) were exhibited by 40% concentrations of *A. melegueta* and *A. danielli* respectively, while the lowest inhibitions were produced by 10% *T. tetraptera* (Figure 2).

The percent inhibitions exhibited by powdered forms of *A. danielli, A. melegueta* and *T. tetraptera* against the three test organisms are as shown in Figure 3. The highest inhibitions (72, 71.06, and 62.48%) were exhibited by *A. danielli* at 20% concentration against *A. flavus, A. versicolor* and *A. ochraceous* respectively. However, percent growth inhibitions exhibited by 5, 10 and 20% concentrations of the same botanical against each of the three test fungi were not significantly different (P ≤ 0.05) from one another (Figure 3). The lowest percent inhibitions, 3.51, 8.84 and 3.25% were exhibited by 5% *T. tetraptera* against *A. flavus, A. versicolor* and *A ochraceous* respectively. These were also not significantly different (P ≤ 0.05) from the inhibitions induced by 10 and 20% concentrations of the same extract against the three fungi isolates.

As shown in Figure 4, the percent inhibition ranges of 53.91 - 56.79%, 54.89 – 57.14%, 41.05 – 46.59% and 50.50 – 53.05% were recorded for the respective combinations of *A. danielli* and *A. melegueta* (D+M), *A. danielli* and *T. tetraptera* (D+T), *A. melegueta* and *T. tetraptera* (M+T) and a combination of *A. danielli, A. melegueta* and *T. tetraptera* (D+M+T) against *A. flavus.* There was however no significant difference (P ≤ 0.05) among the percentage inhibitions produced by the extract combinations against the fungus. While the lowest and highest percent inhibitions exhibited against *A. versicolor* by 5% M+T and 20% D+T were 39.29

ISSN: 0975 – 8704
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and 56.63% respectively, there was no significant difference ($P \leq 0.05$) in the inhibitions exhibited when compared with the concentrations of the remaining combinations (Figure 4).

Except for 20% D+T and 5% M+T which exhibited highest and lowest percent inhibitions of 53.34 and 32.59% respectively against *A. ochraceous,* concentrations of other powder extract combinations induced percent inhibitions that were not significantly different ($P \leq 0.05$) from one another (Figure 4).

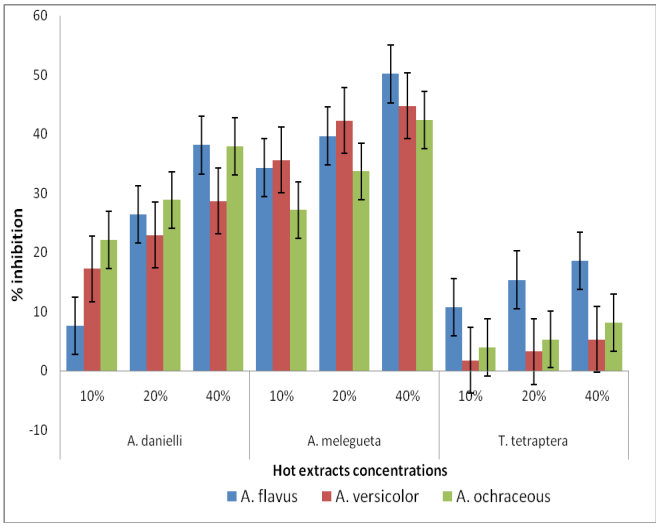

Figure 1: Effects of hot water extracts of *Aframomum danielli, A. melegueta* and *Tetrapleura tetraptera* on mycelial growth of *Aspergillus flavus, Aspergillus versicolor* and *Aspergillus ochraceous*

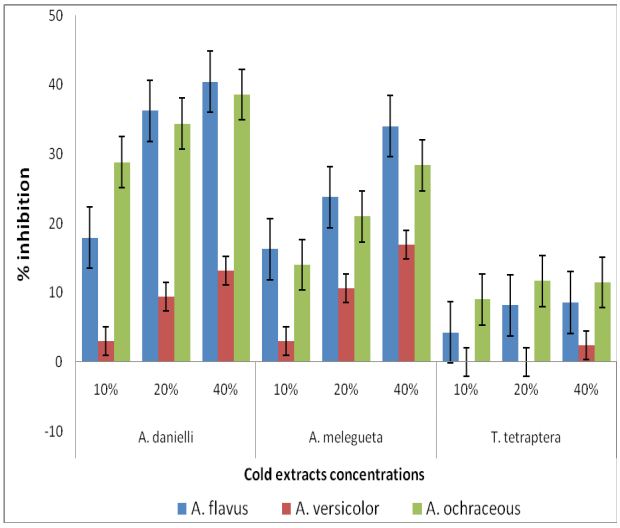

Figure 2: Effects of cold water extracts of *Aframomum danielli, A. melegueta* and *Tetrapleura tetraptera* on mycelial growth of *Aspergillus flavus, Aspergillus versicolor* and *Aspergillus ochraceous*

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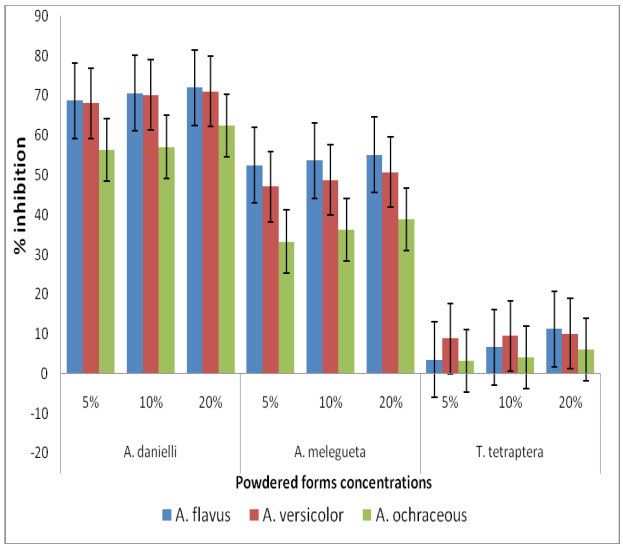

Figure 3: Effects of powdered forms of *Aframomum danielli, A. melegueta* and *Tetrapleura tetraptera* on mycelial growth of *Aspergillus flavus, Aspergillus versicolor* and *Aspergillus ochraceous*

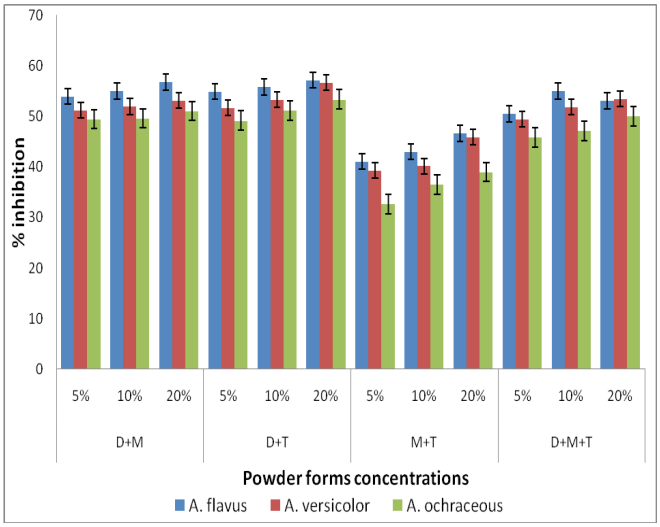

Figure 4: Effects of combined powdered forms of *Aframomum danielli* (D), *A. melegueta* (M) and *Tetrapleura tetraptera* (T) on mycelial growth of *Aspergillus flavus, Aspergillus versicolor* and *Aspergillus ochraceous*

Discussion

Medicinal and aromatic plants (spices) have been shown to possess medicinal value, in particular, anti-microbial activity. Most of these spices contain compounds like alkaloids, tannins, saponin, steroids, glycosides, flavonoid, terpenoids and phenol, the presence of which support their use as antimicrobial agents. *Aframomum spp.* are major food plants and their antimicrobial properties have been reported [12]. While bioassays of the extract of *A. danielli* have revealed active growth inhibitors of *Salmonella enteriditis, Pseudomonas fragi*, *P. flourescens, Proteus vulgaris, Streptococcus pyogens, Staphylococcus aureus, Aspergillus flavus, A. parasiticus, A. ochraceus* and *A. niger* [13], pods of *Tetrapleura tetraptera* have been found to possess some anti-microbial properties which suggest the potential use of its extract for reducing the growth of pathogens in food systems [14]. Results obtained from the use of various aqueous

ISSN: 0975 – 8704
IJBLST (2016), 8(3):16-22

extracts and powdered forms of *A. danielli, A. melegueta* and *T. tetraptera* in the control of storage moulds, *A. flavus, A. ochraceous* and *A. versicolor* in this study clearly revealed their varying anti-microbial properties.

While assessing the anti-fungal effects of cold and hot water extracts of cardamom, chilli, coriander, onion, garlic, ginger, and galangale against the three Roselle pathogens *Phoma exigua, Fusarium nygamai* and *Rhizoctonia solani*, [15] discovered that all the seven spices showed significant anti-fungal activity at three concentrations (10, 20 and 30% of the crude extract) *in-vitro.* The cold water extract of garlic exhibited good anti-fungal activity against all three tested fungi. In the case of the hot water extracts, garlic and ginger showed the best anti-fungal activity. Of the two extraction methods, cold water extraction was generally more effective than hot water extraction in controlling the pathogens. Findings from this study has revealed that hot water extracts of *A. melegueta* were more effective against *A. flavus, A.versicolor* and *A. ochraceous* than their cold water extracts, while the reverse was the case for *A. danielli* with the exception of *A. versicolor* against which a higher (hot water extract) effectiveness was noticed.

In a research aimed at controlling the storage moulds and weevils affecting maize grains with the aid of powdered forms of bush pepper *(Piper guineensis)*, [16] noticed a very significant reduction in the number of infected seeds in the treated seed lot when compared with the untreated control. The scientists also observed that the higher the powder extract concentration, the higher its effectiveness. It was therefore concluded that *P. guineensis* seed powder extract has the ability to prevent maize seeds from being infected by storage fungi. Results obtained from this study revealed that except for the inhibitions produced by *Tetrapleura tetraptera* against *A.flavus* and *A. ochraceous*, the powder extracts of the three spices used were appreciably more effective than either of their respective cold and hot water extracts. The percent inhibitions exhibited by each of these powdered forms also increased with concentration.

There was no noticeable synergistic effect against any of the test moulds when at least two of the powder extracts were combined. The combination of either or the duo of *A. danielli* and *A.melegueta* with *T. tetraptera* (which was the least effective among the three) only improved the latter's effectiveness. In contrast to results from this study, [17] observed that the combination of *X. aethiopica* and *P. guineense* which when used alone did not produce a strong fungistatic effect, gave an apparent synergistic suppression of *A. flavus* growth.

Conclusion/Recommendation

It has however been discovered that ground powders, rather than hot or cold water extracts of *A. danielli* and *A. melegueta* can effectively control storage moulds of cocoa. Neither of the aqueous nor powdered forms of *T. tetraptera* can effectively control the organisms. Combinations of at least two of the powders tested (although not synergistic) can also control the pathogens to some extent.

In addition to the proper handling of cocoa beans right from the field down to the store (which will help reduce storage fungi population), ground powders of *A. danielli* and *A. melegueta* can be used to effectively curb the proliferation of the implicated moulds and thus prevent the attendant health hazards.

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